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Evaluating lactoferrin and calprotectin as markers of intestinal inflammation in chronic pancreatitis

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HIGHLIGHTS

- Exploration of intestinal inflammation in chronic pancreatitis patients with altered bowel habits.
- Assessment of 23 patients using lactoferrin and calprotectin as intestinal inflammation biomarkers.
- Intestinal inflammation was detected in all patients; positive correlation between both biomarkers.
- Established connection between altered bowel habits and intestinal inflammation in chronic pancreatitis.

ABSTRACT – **Background** – The treatment of chronic pancreatitis does not consistently solve intestinal abnormalities, and despite the implementation of various therapeutic measures, patients often continue to experience persistent diarrhea. Therefore, it is imperative to recognize that diarrhea may stem from factors beyond pancreatic insufficiency, and intestinal inflammation emerges as a potential contributing factor. **Objective** – The aim of this study was to assess fecal lactoferrin and calprotectin levels as indicators of intestinal inflammation in patients with chronic pancreatitis experiencing persistent diarrhea. **Methods –** In this study, 23 male patients with chronic pancreatitis primarily attributed to alcohol consumption and presenting with diarrhea (classified as Bristol stool scale type 6 or 7), underwent a comprehensive evaluation of their clinical and nutritional status. Fecal lactoferrin and calprotectin levels were measured utilizing immunoassay techniques. **Results –** The average age of the participants was 54.8 years, 43.5% had diabetes, and 73.9% were smokers. Despite receiving enzyme replacement therapy and refraining from alcohol for over 4 years, all participants exhibited persistent diarrhea, accompanied by elevated calprotectin and lactoferrin levels indicative of ongoing intestinal inflammation. **Conclusion –** The findings of this study underscore that intestinal inflammation, as evidenced by elevated fecal biomarkers calprotectin and lactoferrin, may contribute to explaining the persistence of diarrhea in patients with chronic pancreatitis.

Keywords – Biomarkers; fecal biomarkers; lactoferrin; calprotectin; exocrine pancreatic insufficiency; chronic pancreatitis; intestinal inflammation; diarrhea.

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INTRODUCTION

Chronic pancreatitis (CP) is characterized by a progressive inflammatory process that leads to fibrosis and calcification of the pancreas[1]. This results in irreversible morphological changes, loss of pancreatic function with impairment of nutrient absorption and digestion (exocrine function), and insulin production (endocrine function). The main etiology of the disease is alcohol consumption, responsible for over 70% of cases[1-4]. In an epidemiological study of CP in Brazil, alcohol accounts for roughly 90% of the etiology[5].

Diagnosis of CP is based on clinical and laboratory findings and imaging studies[3,6]. The primary manifestations of pancreatic insufficiency include poor digestion, abdominal pain, and nutritional abnormalities[1,3,4,7]. Fat malabsorption can lead to steatorrhea, weight loss, flatulence, deficiencies in fat-soluble vitamins (A, D, E, and K), and deficiencies in B-complex vitamins[4,8,9]. Furthermore, endocrine insufficiency, resulting from the destruction of the Islets of Langerhans, is associated with type 3c diabetes in nearly 10% of patients with alcoholic pancreatitis[1].

Treatment of patients with CP is centered on nutritional counseling, correction of metabolic abnormalities, and in cases of pancreatic steatorrhea, appropriate oral replacement of pancreatic enzymes[4,9]. For patients with pancreatic enzyme replacement therapy (PERT) who continue to experience symptoms, additional management options to consider include escalation of PERT dose, addition of an H2-blocker, or a proton pump inhibitor (if not currently using)[9-11]. Patients should also abstain from alcohol consumption and, for smokers, cease smoking. Insulin treatment of type 3c diabetes should be administered when indicated[1,7,9]. Supplementation of calcium, magnesium, zinc, thiamine, folic acid, fat-soluble vitamins (A, D, E, and K), and B12 depends on the specific deficiencies identified through blood measurements[9-13].

Despite treatment with clinical, nutritional, and pharmacological interventions, patients with CP can exhibit gastrointestinal disturbances, with persistent diarrhea not explained by exocrine or endocrine insufficiency. In such cases, intestinal inflammatory activity may be suspected and could be detected by intestinal inflammatory biomarkers[14].

Numerous studies have identified a range of fecal markers and intestinal mucosal biomarkers as indicators of disease activity. These include various biomarkers of inflammatory bowel disease found in feces, such as calprotectin, calgranulin C, lactoferrin, cathelicidins, osteoprotegerin, beta-glucuronidase, and neutrophil gelatinase-associated lipocalin[15]. These non-invasive biomarkers have been studied to enhance the diagnostic accuracy for detecting intestinal inflammatory activity[16-18]. Lactoferrin and calprotectin, both neutrophil-derived, are widely recognized as efficient biomarkers for inflammation in patients with intestinal inflammatory diseases. Additionally, they are cost-effective and straightforward to perform[17,19].

Considering these features, both markers could be beneficial for detecting the presence of intestinal inflammatory activity in patients with CP presenting persistent diarrhea. However, limited research has been conducted on evaluating these biomarkers in CP patients who continue to experience intestinal abnormalities even before addressing the other potential causes of diarrhea.

The objective of this study is to assess the occurrence of intestinal inflammatory activity in a group of CP patients who exhibited persistent intestinal abnormalities, using fecal biomarkers associated with inflammation.

METHODS

This study was prospective and longitudinal in nature, targeting consecutive patients aged 18 years and older who presented with CP and intestinal abnormalities at the outpatient clinic of Hospital Sao Paulo, Federal University of São Paulo, Brazil. The CP diagnosis was confirmed through a combination of clinical assessments, laboratory tests, and imaging studies. However, patients were excluded if they had undergone intestinal resection, had cancer or parasitosis, suffered from intestinal inflammatory diseases or infectious diarrhea, or if they were taking anticoagulants, antibiotics, or anti-inflammatory medication.

The study's protocol received approval from the local Ethics Committee of Hospital Sao Paulo (appro-

val number 493.024 on December 13, 2013). All participants were thoroughly informed about the study's nature and provided their signed informed consent before participation.

At the first visit, each patient underwent a detailed clinical and nutritional evaluation. Blood samples were collected for a series of tests, including complete blood count, erythrocyte sedimentation rate, C-reactive protein levels, hepatitis B and C viruses (HBV and HCV) and human immunodeficiency virus (HIV) serology, glucose levels, glycated hemoglobin, albumin, total cholesterol along with its fractions, triglycerides, iron, ferritin, and transferrin levels. Additionally, to exclude specific intestinal conditions, a stool sample was collected for microbiological analysis. This analysis checked for the presence of eggs, larvae, or cysts, cultures for enteropathogens, and included a Sudan III stain test to evaluate fecal fat content.

Bristol stool scale

The Bristol Stool Scale, developed by Heaton et al.[20], is a widely recognized medical tool used to categorize human feces into seven distinct types. This tool is extensively used both in clinical settings and for research purposes[21]. It involves an individual examining their stool and then comparing it to the scale's detailed descriptions and visual illustrations. Based on the closest match, the stool is classified into one of the seven defined categories, as shown below:

Type 1: Separate hard lumps, like nuts (hard to pass).
Type 2: Sausage-shaped but lumpy.
Type 3: Like a sausage but with cracks on the surface.
Type 4: Like a sausage or snake, smooth and soft (considered ideal).
Type 5: Soft blobs with clear-cut edges (passed easily).
Type 6: Fluffy pieces with ragged edges, mushy.
Type 7: Watery, no solid pieces, entirely liquid.

Specifically, types 1 and 2 on the scale are indicative of constipation, while types 6 and 7 suggest the presence of diarrhea.

Follow-up of patients

Over a period of 12 months, the patients in the study were closely monitored and provided with comprehensive support, including nutritional advice, clinical care, and pharmacological treatment using pancreatic enzyme replacement therapy (PERT). Their baseline health characteristics were recorded and then compared with data collected at the end of the 12-month follow-up period. At the end of the 12-month study period, a stool sample was obtained from each patient to conduct immunoassays, which included tests for fecal elastase, calprotectin, and lactoferrin.

Quantitative calprotectin determination

Fecal samples were thawed, and the test was conducted according to the manufacturer's instructions (Calprotectin test – NovaTec, Germany). The test is based on an immunosorbent assay using a polyclonal antibody against calprotectin. The mean values of optical density were read in duplicates using a 450 nm filter. A control curve was constructed with different concentrations of calprotectin. Optical density (OD) values of the patient's samples were plotted on the curve, and the calprotectin values obtained were expressed in ng/mL. The normal value of calprotectin in healthy individuals without intestinal inflammation is up to 50 ng/mL[22].

Qualitative lactoferrin test

Fecal samples were thawed, and the test was conducted according to the manufacturer's instructions (IBD-CHEK® T5008 – TechLab, Inc. USA). The IBD-CHEK® system uses immobilized polyclonal antibodies against lactoferrin. The detecting antibody consists of a polyclonal antibody conjugated to horseradish peroxidase. Following the addition of the substrate, a color change is detected due to the enzyme-antibody-antigen complexes that form in the presence of lactoferrin. The samples were read using an ELISA reader with a 450 nm filter. Values of optical density less than or equal to 0.200 were considered negative, and those greater than 0.200 were considered positive[23].

Quatitative fecal elastase test

Elastase-1 concentration was determined by the ELISA method (Pancreatic Elastase 1TM Stool Test; ScheBo® Biotech, Giessen, Germany), using two spe-

cific monoclonal antibodies against human pancreatic elastase-1 that bind to two different epitopes. The concentration of elastase-1 (µg/g of feces) was determined by photometry. The lower limit of detection was 1 ng/mL. Mild to moderate pancreatic insufficiency was defined as an elastase-1 concentration of <200 µg/g of feces, and a concentration of <100 µg/g of feces was considered severe insufficiency[24].

Statistical analysis

Descriptive analysis included median, interquartile ranges, and frequencies. The significance level was defined as 0.05 (5%). Non-parametric tests were applied for comparison among groups (Mann-Whitney and Kruskal-Wallis). To verify the correlation between calprotectin and lactoferrin, the Spearman's correlation coefficient was applied. The receiver operating characteristic curve (ROC curve) and area under the ROC curve (AUROC) were constructed to discriminate between these two biomarkers (lactoferrin and calprotectin) and identify the one that simultaneously optimizes the best sensitivity and specificity values. The statistical analyses were performed using SPSS version 17 – IBM, Minitab 16, and Microsoft Excel.

RESULTS

In this study, we included a total of twenty-three patients who exhibited intestinal abnormalities. These patients were specifically classified as having diarrhea according to the Bristol Stool Form Scale, which categorizes it as either type 6 or type 7.

Baseline clinical and laboratorial characteristics of patients

All patients were males with a median age of 53 years. The underlying cause of their CP was alcohol abuse, with a median duration of 25 years. Computed tomography scans revealed morphological abnormalities consistent with CP in all patients, characterized by pancreatic atrophy in 91.3% of cases, diffuse calcification in 65.2%, localized calcification in 8.7%, diffuse ductal dilatation in 52.2%, focal ductal dilatation in 13%, and 17.4% of patients showed signs of an inflammatory focal mass.

Enzymatic replacement therapy was being used by 17 patients (73.9%), and 19 out of 23 patients (82.6%) had abstained from alcohol for more than 4 years. Regarding comorbidities, 10 patients (43.5%) were diagnosed with diabetes, while 17 (73.9%) were active smokers. Other comorbidities included arterial hypertension (47.8%), obesity (17.4%), chronic liver disease (17.4%), and cardiomyopathy (17.4%). The nutritional evaluation revealed that most patients (n=11) were eutrophic, two patients were underweighted, six were overweight, and four were obese. Detailed clinical and laboratory baseline characteristics can be found in TABLES 1 and 2.

TABLE 1. Clinical characteristics of the chronic pancreatitis patients evaluated (N=23).

Parameters	Percentiles			Min	Max	CI
	25	Median	75			
Age (years)	50.0	53.0	60.0	33.0	84.0	4.4
Time of alcohol abuse (years)	13.0	25.0	32.0	10.0	39.0	3.8
Time of CP diagnosis (years)	4.0	7.0	13.0	1.00	21.00	2.57
Amount of alcohol (g/day)	100	115.0	340.0	40.0	600.0	65.2
Smoking time (years)	0.0	25.0	38.0	0.0	52.0	6.8
Time of DM diagnosis (years)	0.0	0.0	11.0	0.00	22.00	3.03
Daily bowel movements	1.0	2.0	2.0	1.0	4.0	0.3
Time on enzymatic replacement (years)	1.0	4.00	6.0	0.00	6.00	1.00

CP: chronic pancreatitis; DM: diabetes mellitus; Min and Max: minimum and maximum values of the sample; CI: confidence interval.

TABLE 2. Laboratorial parameters of the chronic pancreatitis patients evaluated (N=23).

Parameters	Percentiles			Min	Max	CI
	25	Median	75			
Eritrocytes (millions/μL)	4.2	4.8	5.0	3.2	5.3	0.2
Hematocrit	41.2	43.0	45.7	28.0	48.6	1.8
Hemoglobin	13.8	14.8	15.5	8.9	15.9	0.7
Leucocytes (/L)	5,900	6,990	8,880	4.940	12.900	807
Platelets (X10^3/μL)	167.0	203.0	250.0	130.0	313.0	20.5
Erythrocyte sedimentation rate (mm)	12.0	13.0	15.0	6.0	37.0	2.5
Reactive C protein (mg/L)	2.4	4.3	5.1	0.9	6.5	0.7
Blood glucose (mg/dL)	97.0	108.0	152.0	82.0	548.0	41.9
Glycated hemoglobin (%)	5.5	6.4	8.7	4.1	13.7	1.0
Albumin (g/dL)	4.1	4.5	4.7	3.1	5.4	0.2
Cholesterol (mg/dL)	143.0	160.0	195.0	91.0	346.0	25.5
Triglycerides (mg/dL)	82.0	92.0	190.0	34.0	1349.0	107.7
Iron (ng/mL)	78.0	112.0	125.0	11.0	178.0	17.2
Transferrin (mg/dL)	201.0	233.0	254.0	145.0	317.0	15.0
Ferritin (ng/mL)	58.0	78.0	167.0	9.0	890.0	78.8
Lactoferrin (OD)	0.05	0.32	0.51	0.04	0.99	0.12
Calprotectin (ng/dL)	312	691	798	283	1100	108.9

Min and Max: minimum and maximum values of the sample; CI: confidence interval; OD: optical density.

Follow-up of studied patients

Despite 12 months of receiving comprehensive care including nutritional support, enzymatic replacement therapy, and diabetes management, all patients in the study still experienced intestinal abnormalities. These abnormalities were marked by stools of soft, pasty consistency, or even liquid diarrhea. However, steatorrhea was not observed in any of the patients, as confirmed by the universally negative Sudan III stain tests for fecal fat.

Additionally, there was no reported weight gain in any of the patients throughout the follow-up period. TABLE 3 provides a comparison of the nutritional baseline characteristics of the patients with the data collected at the end of the 12-month follow-up.

Fecal biomarkers of intestinal inflammation

The fecal biomarkers of intestinal inflammation were evaluated in samples collected at the end of the follow-up (TABLE 2). Lactoferrin (considered positive if OD ≥0.200) and calprotectin (considered positive if ≥50 ng/dL) presented results compatible with intestinal inflammation, with median fecal levels above the normal limits (0.320 OD and 691 ng/dL, respectively). All patients showed evidence of severe exocrine pancreatic insufficiency with very low levels of fecal elastase-1, median value 12.0 μg/g feces.

There was no association between the intestinal inflammation markers and the presence of diabetes, active alcohol intake, enzymatic replacement therapy, or tobacco use. Lactoferrin and calprotectin

TABLE 3. Nutritional characteristics at baseline and after 12 months of follow-up.

Parameters	Initial			Final			*P*
	Percentiles			Percentiles			
	25	Median	75	25	Median	75	
Weight (kg)	59.0	70.0	75.0	56.7	65.5	79.0	>0.05
Body Mass Index (BMI)	20.6	24.0	26.4	19.5	22.6	27.6	>0.05
Sum of the 4 skinfolds*	28.5	38.0	54.0	26.0	40.5	58.5	>0.05
Body fat 4 in the site skinfolds (%)*	17.7	20.8	25.9	15.6	22.9	27.9	>0.05

*The 4 Skinfolds (mm – millimeters): triceps skinfold; bicipital skinfold; subscapular skinfold and suprailiac skinfold[37].

showed a very good positive correlation (R=0.87; *P*<0.001): the higher the fecal calprotectin value, the higher the fecal lactoferrin value. All patients had positive fecal calprotectin, whereas only 65.2% of patients had positive fecal lactoferrin (defined as OD >0.200). A ROC curve was constructed to determine a positive lactoferrin result (OD levels >0.200) based on the best calprotectin cut-off point, optimizing sensitivity and specificity (AUROC=1.000). The point that optimizes sensitivity and specificity was 478 ng/mL. Thus, values above 478 ng/mL of calprotectin have 100% sensitivity and specificity for the diagnosis of a positive lactoferrin.

DISCUSSION

In this study, our objective was to assess the occurrence of intestinal inflammatory activity in a group of CP patients who exhibited persistent intestinal abnormalities according to the Bristol Stool Scale after the correction of the main causes of diarrhea. This approach would help to understand the gastrointestinal symptoms associated with chronic pancreatitis more comprehensively[25]. This included pancreatic enzyme replacement therapy (PERT) for those with steatorrhea and pharmacological treatments for diabetic patients, alongside adherence to alcohol abstinence and receiving nutritional support.

Despite ongoing treatment for associated conditions, patients continued to exhibit bowel habit abnormalities throughout the 12-month follow-up. This study investigated intestinal inflammation as a potential underlying cause. Fecal analyses, including microbiological tests and fecal fat assessments, were negative. However, end-of-study measurements of fecal biomarkers, specifically lactoferrin and calprotectin, revealed elevated levels in all patients, indicating the presence of intestinal inflammation.

Both calprotectin and lactoferrin are recognized as sensitive markers for inflammation, though their non-specific nature warrants careful interpretation[17,18,26-28]. Their elevation in CP patients, as observed in this study, could be indicative of an inflammatory process within the intestinal tract.

The reasons of the process of intestinal inflammation in CP patients are not fully understood. Some causes have been proposed in the literature, such as malnutrition, dysbiosis[29], and the use of high doses of PERT[30]. This association of intestinal inflammation in patients with chronic pancreatitis have been described mainly in patients with cystic fibrosis[31] but is possible to occur also in patients with other causes of CP[32].

Fecal calprotectin, especially, has shown high sensitivity in this study, being elevated in all patients. Its role as a non-invasive marker is further strengthened, offering a valuable tool for initial screening of intestinal inflammation in CP patients.

The absence of serum inflammatory markers could be explained by the presence of advanced chronic pancreatitis is most patients, characterized by fibrosis with no inflammation.

Although CP could be a contributing factor to the increased levels of these biomarkers, it's crucial to consider other potential causes of intestinal inflammation. These could include co-existing conditions like small intestinal bacterial overgrowth (SIBO), lactose intolerance, celiac disease, blind loop syndrome, or other gastrointestinal conditions[2,33-35]. These factors might explain the inadequate response to therapy interventions in CP patients with persistent intestinal abnormalities[2,35]. The study, however, did not extensively investigate these alternative explanations, which leaves room for further research.

The study's limitations include its narrow focus on CP patients without thoroughly investigating other potential causes of diarrhea and intestinal inflammation. The lack of a control group comprising asymptomatic individuals, patients with asymptomatic chronic pancreatitis or patients with other gastrointestinal disorders limits the ability to definitively attribute the elevation of these biomarkers solely to CP.

These findings indicate that fecal calprotectin could be an effective initial screening tool for intestinal inflammation in CP patients, before undergoing more invasive and expensive procedures such as colonoscopy and biopsy. If these results are confirmed, fecal calprotectin could serve as a valuable non-invasive biomarker for CP patients presenting changes in bowel habits, particularly those without steatorrhea[17,18,36].

In conclusion, the study highlights a significant correlation between CP and elevated levels of fecal calprotectin and lactoferrin, suggesting a direct link

to intestinal inflammation. However, the presence of other gastrointestinal disorders as contributing factors cannot be ruled out. Further research is warranted to explore these associations more comprehensively.

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Authors' contribution

Carvente CT contributed to data collection, survey execution, statistical analysis, interpretation of results, and manuscript writing. Ferraz MLCG contributed to the conception of the study design, discussion of results, writing, translation, and manuscript revision. Toledo CF also contributed to the conception of the study design, discussion of results, writing, and manuscript revision.

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Carvente CT, Ferraz MLCG, Toledo CF. Avaliação da lactoferrina e da calprotectina como marcadores de inflamação intestinal na pancreatite crônica. Arq gastroenterol. 2024;61:e24003.

RESUMO – Contexto – O tratamento da pancreatite crônica não resolve de forma consistente as anomalias intestinais e, apesar da implementação de várias medidas terapêuticas, os pacientes muitas vezes continuam a apresentar diarreia persistente. Portanto, é imperativo reconhecer que a diarreia pode resultar de fatores além da insuficiência pancreática, e a inflamação intestinal surge como um potencial fator contribuinte. **Objetivo –** O objetivo deste estudo foi avaliar os níveis fecais de lactoferrina e calprotectina como indicadores de inflamação intestinal em pacientes com pancreatite crônica com diarreia persistente. **Métodos –** Neste estudo, 23 pacientes do sexo masculino com pancreatite crônica atribuída principalmente ao consumo de álcool e apresentando diarreia (classificada na escala de fezes de Bristol tipo 6 ou 7), foram submetidos a uma avaliação abrangente de seu estado clínico e nutricional. Os níveis fecais de lactoferrina e calprotectina foram medidos utilizando técnicas de imunoensaio. **Resultados –** A idade média dos participantes foi de 54,8 anos, 43,5% tinham diabetes e 73,9% eram fumantes. Apesar de receber terapia de reposição enzimática e abster-se de álcool por mais de 4 anos, todos os participantes apresentaram diarreia persistente, acompanhada por níveis elevados de calprotectina e lactoferrina, indicativos de inflamação intestinal contínua. **Conclusão –** Os achados deste estudo ressaltam que a inflamação intestinal, evidenciada pelos biomarcadores fecais elevados calprotectina e lactoferrina, pode contribuir para explicar a persistência da diarreia em pacientes com pancreatite crônica.

Palavras-chave – Biomarcadores; biomarcadores fecais; lactoferrina; calprotectina; insuficiência pancreática exócrina; pancreatite crônica; inflamação intestinal; diarreia.

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